Testosterone and Dihydrotestosterone in Newborn Hospitalized and Healthy Foals

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Abstract

Steroidogenesis, a process by which steroid hormones are produced through enzymatic conversion of cholesterol occurs in many endocrine organs, including the adrenal gland, gonads, and placenta. Sex steroids include progestogens, androgens, and estrogens. In the equine fetus, androgens such as testosterone and dihydrotestosterone (DHT) are produced in the zona reticularis of the foal's adrenal gland and the equine placenta through the metabolism of steroid precursors. Progesterone, a steroid hormone associated with pregnancy in people and animals, has been associated with disease severity in critically ill foals. However, the relationship between androgens (testosterone, DHT) with disease in neonatal foals has not been evaluated. Therefore, the objective of this study was to determine if androgen concentrations were associated with illness in newborn foals. This information could enhance our understanding of endocrine changes in the newborn foal as well as changes in placental function at the end of pregnancy.

The study included healthy (n=13), sick non-septic (SNS; n=27), and septic (n=23) newborn foals. Septic foals are those with a sepsis score ≥12 and evidence of bacterial infection; SNS foals were those with a sepsis score ≤11 and no evidence of bacterial infection. Blood samples were collected at admission (time 0), 24, 48, and 72 hours, centrifuged, and serum aliquoted and stored in a -80°C until analysis. Hormone concentrations were measured through immunoassays. Clinical, laboratory, and endocrine data were evaluated for normality and analyzed with parametric and non-parametric statistical tests.

In healthy foals, testosterone and DHT concentrations showed a steady decrease over 72 hours. In contrast, septic foals had increased testosterone and DHT concentrations at time 0

compared to healthy foals that remained elevated during hospitalization. Similarly, testosterone and DHT concentrations were higher in foals that died. In SNS foals, androgens followed a similar pattern to healthy foals.

These results indicated that severe infections alter the steroid endocrine balance in sick newborn foals, which was evident by a delayed return of androgens to values similar to healthy foals over time. Our findings have provided additional understanding of diseases affecting the equine neonate.

Table of Contents

Introduction

Steroidogenesis leads to the production of many hormones. This occurs in the adrenal glands, gonads, and placenta. The process begins with conversion of cholesterol to pregnenolone by a side-chain cleavage (Auchus and Miller, 2016). The cleavage occurs in the mitochondria and endoplasmic reticulum of the cell through the use of cytochrome P450 enzyme (Auchus and Miller, 2016). Within fetal equine circulation, it is believed that the gonads and adrenal glands are the primary source of dehydroepiandrosterone (DHEA) and pregnenolone (Legacki et al., 2017). Tissue specific enzyme pathways will lead to the conversion of pregnenolone to other steroid hormones (Fails and Magee, 2018).

The adrenal glands are endocrine organs located cranial to the kidneys, with a cortex and medulla, which produce different hormones (steroids, catecholamines). The innermost cellular zone of the adrenal gland cortex is the zona reticularis. While this zone produces some glucocorticoids such as cortisol, it predominately produces androgens such as DHEA that is also a precursor to other steroids (Hughes and Chatterjee, 2016). In foals, most androgens originate from DHEA. It is likely that DHEA production in the fetus and newborn foal come from the gonads (Legacki et al., 2017).

In 1991, the American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference met to determine definitions relating to sepsis. Sepsis can be defined as a systemic inflammatory response syndrome (SIRS) induced by infection. Sepsis is a systemic inflammatory response to severe clinical insults demonstrated by two or more of the following conditions (1) fever or hypothermia; (2) tachycardia; (3) tachypnea or hypocapnia; (4) leukopenia, leukocytosis, or increased circulating immature neutrophils. Over time, additional

terms have been added (Bone et al., 1992). In foals, early diagnosis and treatment of sepsis are required for a favorable outcome. A previous sepsis scoring method indicated that a score of 15 or higher indicated possible septicemia or infection; however, a score of ≥12 resulted in higher sensitivity and specificity (Brewer and Koterba, 1988)

Sepsis is the main cause of death in equine neonates (Dembek et al., 2013). These septic foals are likely to have increased concentrations of adrenocorticotropic hormone (ACTH) and ACTH:cortisol ratios (Gold et al., 2007). High ACTH:cortisol ratios have been associated with higher mortality, laboratory abnormalities, and altered concentrations of several adrenocortical steroids (Dembek et al., 2017). In addition to cortisol and ACTH, other adrenocortical products, like progesterone have been found to be significantly associated with mortality (Dembek et al., 2017). The relationship between androgens such as testosterone and dihydrotestosterone have not been evaluated in healthy and sick neonatal foals. Therefore, the objective of this study was to determine if androgen concentrations were associated with illness in newborn foals. Our hypothesis is steroid endocrine imbalances in sick neonatal foals can be seen through altered concentrations of testosterone and dihydrotestosterone.

Materials and Methods

A total of 63 foals $<$ 3 days of age born during the 2018 foaling season were used for this study. The foals were any breed and sex. Sick non-septic (SNS; n=27) and septic (n=23) foals were admitted to either The Ohio State University Veterinary Medical Center or Rood and Riddle Equine Hospital (Lexington, KY). Septic foals were those with a sepsis score (Brewer and

Koterba, 1988) ≥12 and a positive blood culture; SNS foals were those with a sepsis score ≤11 and a negative blood culture. Healthy foals (n=13) were evaluated on farm or the hospital. Foals ≤ 3 days of age with normal physical examination, complete blood count (CBC), serum biochemistry profile, serum immunoglobulin G (IgG) > 800 mg/dL, and sepsis scores < 4 were classified as healthy.

Hospitalized foals (n=50) were further classified as survivors (n=38) or non-survivors (n=12). Foals considered survivors were alive and discharged from the hospitals. Foals considered non-survivor were euthanized or died while at the hospitals. Foals euthanized for monetary reasons were not included in this study

This study was approved by Institutional Animal Care and Use Committee of The Ohio State University (Protocol # 2008A0170-R1) and adhered to the principles of humane treatment of animals in veterinary research, as stated by the American College of Veterinary Internal Medicine and the National Institutes of Health Guidelines.

Blood samples were collected at time 0, 24, 48, and 72 hours. Hospitalized foals received time zero (0) upon admission while blood samples of healthy foals were collected within 72 hours after parturition. Blood in serum tubes were left to clot for 30 minutes before centrifugation. Blood was also collected in EDTA tubes to facilitate collection of serum. The blood was then centrifuged at 2000 g for 10 minutes, aliquoted and stored in -80°C until hormone analysis.

Serum concentrations of testosterone were determined through the use of radioimmunoassay (RIA) (Tecan International, Morrisville, NC). Serum concentrations of DHT were determined through the use of enzyme-linked immunosorbent assay (ELISA) (DRG International, Springfield Township, NJ). Statistical analysis was done through the use of Sigma Plot 14.0 and figures were produced with GraphPad Prism 8.

Results

Testosterone:

Testosterone concentrations decreased from time of zero to time 72 in healthy, SNS, and septic foals (Figure 1A). The levels of testosterone for healthy foals were significantly lower at time 72 in comparison to time zero ($P = 0.003$). Both time 48 and time 72 h were significantly lower than time zero ($P < 0.001$) and time 24 ($P < 0.001$) for foals that were SNS. Septic foals had a significantly lower level at testosterone at time 72 in comparison to time zero ($P = 0.006$).

The concentration of testosterone for survivors and non-survivors also decreased over time (Figure 1B). However, non-survivor testosterone median concentrations were 2.6 times higher (12.2 ng/dL) than survivors (4.7 ng/dL) at time zero. The foals' concentrations of testosterone peaked at time 24 with a median value of 15.7 ng/dL and then began to decrease. There was a much more consistent decrease in concentrations of testosterone for survivor foals. Concentrations of testosterone were significantly lower at time 72 than time zero ($P < 0.001$) and time 24 ($P < 0.001$) in survivor foals. These foals also had significantly lower concentrations at time 48 than time zero ($P < 0.001$) and time 24 ($P < 0.001$).

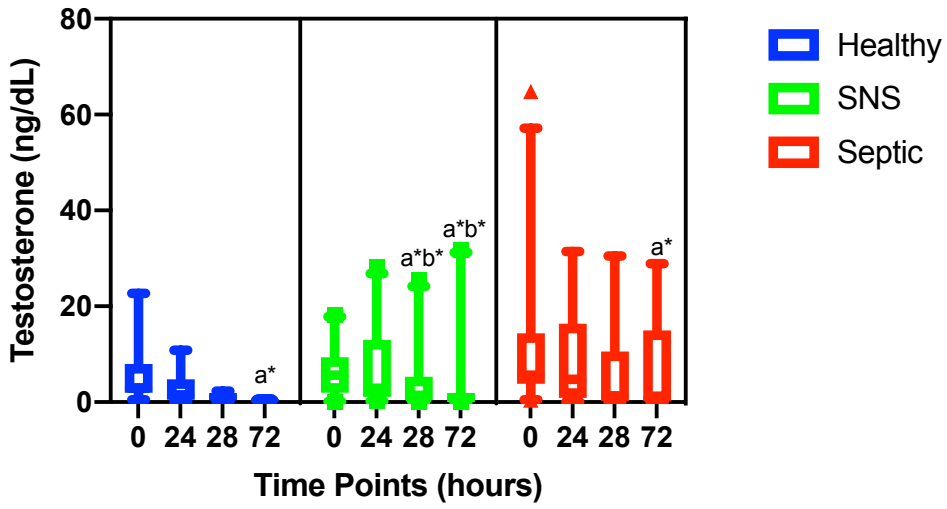
Dihydrotestosterone (DHT):

Similar to testosterone, DHT concentrations decreased from the time of hospital admission or birth to time 72 in healthy and SNS (Figure 2A). In contrast, septic foals had rising levels of DHT until time 24 where it then began to decrease to time 72. Within healthy foals, the concentration of DHT was significantly lower at time 72 than time zero ($P < 0.001$). Concentrations at time 48 were also significantly lower than time 0 ($P = 0.034$). DHT concentrations were significantly lower at time 72 than time zero ($P = 0.02$) and time 24 ($P = 0.004$).

DHT concentrations for survivors and non-survivors followed a similar pattern to that of testosterone concentrations and decreased over time (Figure 2B). Non-survivors had a median concentration about 2.52 times higher (1088.2 pg/mL) than survivors (432.1 pg/mL) at time zero. The concentration of DHT also peaked at time 24 with a median value of 1296 pg/mL and then began to decrease. Within survivor foals, the concentration of DHT was significantly lower at time 72 than time zero ($P = 0.007$) and time 24 ($P = 0.002$). The concentration was also significantly lower at time 48 than time 24 ($P = 0.048$) for survivor foals.

Figure 1: Serum testosterone concentrations in hospitalized and healthy foals over time
Values represented are medians with interquartile ranges. Whiskers represent a 5-95% range.
A) Serum testosterone (ng/dL) concentration of healthy, sick non-septic (SNS), and septic foals
B) Serum testosterone (ng/dL) concentrations of survivor and non-survivor foals
"a" represents a significant difference between time zero within that group
"b" represents a significant difference between time 24 within that group
"*" represents a statistical significance ($P < 0.05$)

A)



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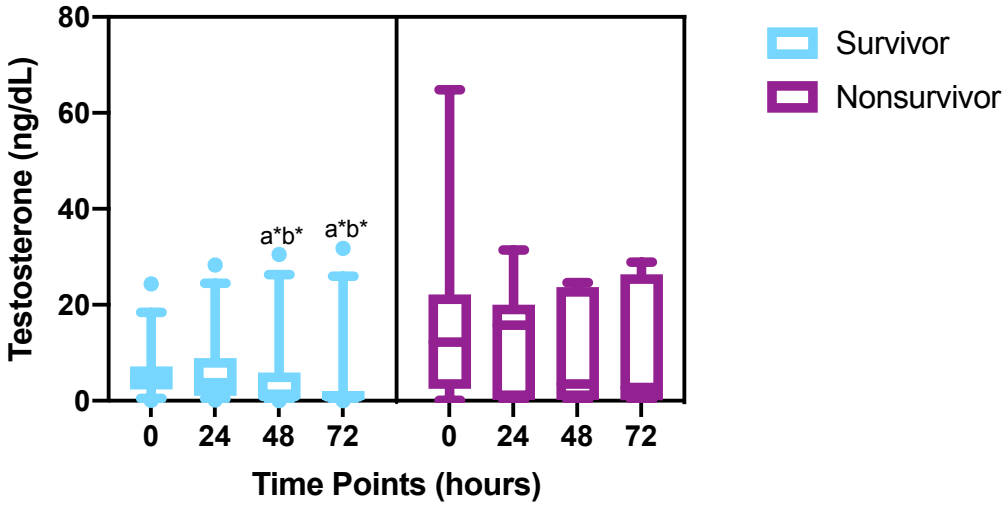
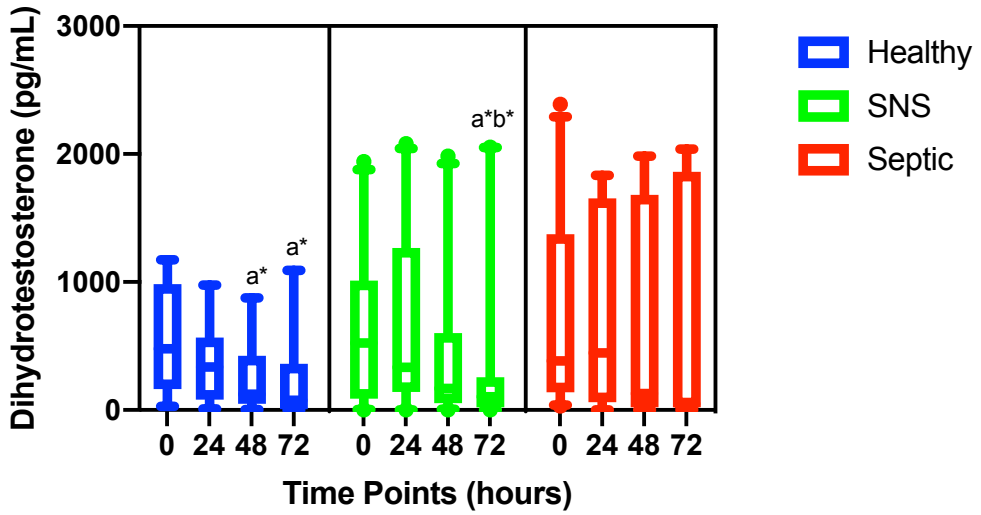


Figure 2: Serum dihydrotestosterone (DHT) concentrations in hospitalized and healthy foals over time

Values represented are medians with interquartile ranges. Whiskers represent a 5-95% range.
A) Serum DHT (pg/mL) concentration of healthy, sick non-septic (SNS), and septic foals
B) Serum DHT (pg/mL) concentrations of survivor and non-survivor foals
"a" represents a significant difference between time zero in that group
"b" represents a significant difference between time 24 in that group
"*" represents a statistical significance ($P < 0.05$)

A)



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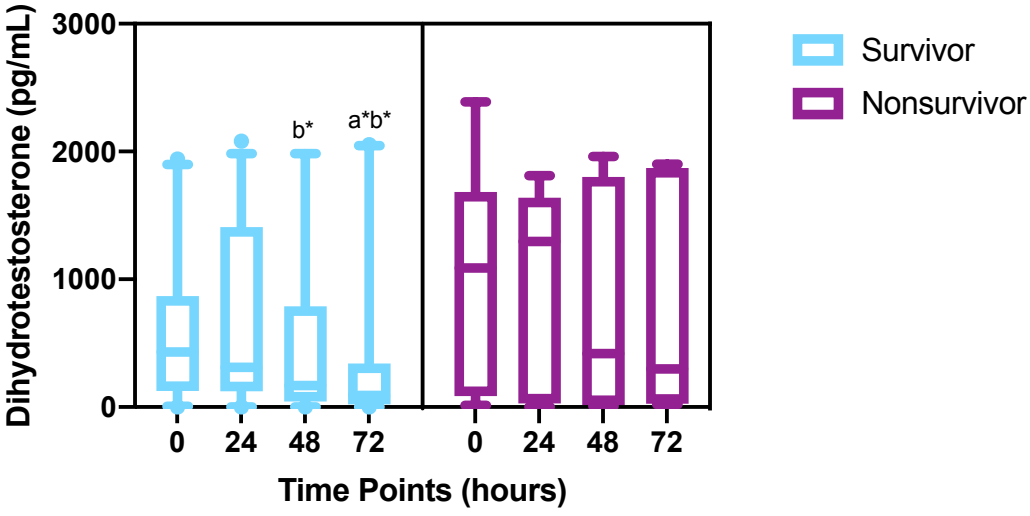

Discussion

A recent study found that plasma steroid concentrations such as pregnenolone, progesterone, deoxycorticosterone (DOC), dehydroepiandrosterone (DHEA), and dehydroepiandrosterone (DHEA-S), decrease over time in healthy neonatal foals (Aleman et al., 2019). Healthy foals have also shown a decrease in pregnane concentrations over the first 48 hours of life (Aleman et al., 2013). Our study also showed that serum testosterone and DHT also decreased in healthy foals over 72 hours from birth. In addition to healthy foals, SNS foals also showed decreased concentrations over time.

Diseased newborn foals have increased levels of progesterone (Dembek et al., 2017) (Aleman et al., 2013), pregnenolone, androstenedione, DHEA, epitestosterone (Aleman et al., 2013) and ACTH (Gold et al., 2007). These studies provide some evidence that sick foals have steroid imbalances and androgens (androstenedione, DHEA) may be altered with disease. While healthy and septic newborn foals had decreasing levels of testosterone and DHT over time, their concentrations from admission to 24 hours were higher in septic than healthy and SNS foals. It was also found that non-surviving foals had higher median concentrations of testosterone and DHT throughout hospitalization in comparison to foals that survived.

Androgen concentrations appear to be linked with disease severity in newborn foals. Steroid metabolism and excretion may play a major role in androgen levels in the equine neonate. In critically ill and non-surviving foals, a delayed decrease of androgen concentration suggests that clearance of androgens may be impaired rather than increased secretion. Additionally, these androgens, like cortisol and progesterone, may have prognostic value.

Further investigation of androgen concentration in healthy and sick newborn foals may be warranted.

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Bibliography

Aleman, M, McCue, PM, Chigerwe, M, and Madigan, JE. Plasma concentrations of steroid precursors, steroids, neuroactive steroids, and neurosteroids in healthy neonatal foals from birth to 7 days of age. *J Vet Intern Med.* 2019; 33: 2286- 2293. https://doi.org/10.1111/jvim.15618

Aleman, M., Pickles, K.J., Conley, A.J., Stanley, S., Haggett, E., Toth, B. and Madigan, J.E. (2013), Neurosteroids in neonatal maladjustment syndrome. Equine Vet J, 45: 661-665. doi:10.1111/evj.12065

Auchus R., and Miller W., Chapter 97 - The Principles, Enzymes, and Pathways of Human Steroidogenesis,Editor(s): J.Larry Jameson, Leslie J De Groot, David M. de Kretser, Linda C. Giudice, Ashley B. Grossman, Shlomo Melmed, John T. Potts, Gordon C. Weir, Endocrinology: Adult and Pediatric (Seventh Edition),W.B. Saunders,2016,Pages 1695-1716.e8,ISBN 9780323189071, https://doi.org/10.1016/B978-0-323-18907-1.00097-4.(http://www.sciencedirect.com/science/article/pii/B9780323189071000974

Bone, Roger C., et al. "Definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis." *Chest*, vol. 101, no. 6, 1992, pp. 1644–1655., doi:10.1378/chest.101.6.1644.

Brewer BD, and Koterba AM. Development of a scoring system for the early diagnosis of equine neonatal sepsis. Equine Vet J. 1988 Jan 1;20(1):18–22.

Dembek, K., Onasch, K., Hurcombe, S., MacGillivray, K., Slovis, N., Barr, B., Reed, S. and Toribio, R. (2013), Renin-Angiotensin-Aldosterone System and Hypothalamic-Pituitary-Adrenal Axis in Hospitalized Newborn Foals. J Vet Intern Med, 27: 331-338. doi:10.1111/jvim.12043

Dembek KA, Timko KJ, Johnson LM, Hart KA, Barr BS, David B, et al. Steroids, steroid precursors, and neuroactive steroids in critically ill equine neonates. Vet J. 2017 Jul;225:42–9.

Fails, Anna Dee, and Christianne Magee. *Anatomy and Physiology of Farm Animals*, John Wiley & Sons, Incorporated, 2018. ProQuest Ebook Central, https://ebookcentral-proquest-com.proxy.lib.ohio-state.edu/lib/ohiostate-ebooks/detail.action?docID=5343190.

Gold JR, Divers TJ, Barton MH, et al. Plasma adrenocorticotropin, cortisol, and adrenocorticotropin/cortisol ratios in septic and normal-term foals. J Vet Intern Med 2007;21:791–796.

Hughes I., and Chatterjee V.K., Chapter 105 - Adrenarche and Adrenopause, Editor(s): J. Larry Jameson, Leslie J De Groot, David M. de Kretser, Linda C. Giudice, Ashley B. Grossman, Shlomo Melmed, John T. Potts, Gordon C. Weir, Endocrinology: Adult and Pediatric (Seventh Edition), W.B. Saunders, 2016,Pages 1833-1840.e3,ISBN 9780323189071,https://doi.org/10.1016/B978-0-323-18907-1.00105-0.

Legacki, Erin L, Barry A Ball, C Jo Corbin, Shavahn C Loux, Kirsten E Scoggin, Scott D Stanley, and Alan J Conley. "Equine fetal adrenal, gonadal and placental steroidogenesis". Reproduction 154.4: 445-454. < https://doi.org/10.1530/REP-17-0239>. vestar